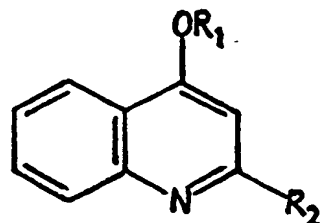




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/47		A1	(11) International Publication Number: WO 95/28929
			(43) International Publication Date: 2 November 1995 (02.11.95)
(21) International Application Number: PCT/GB95/00886 (22) International Filing Date: 20 April 1995 (20.04.95) (30) Priority Data: 9407869.8 20 April 1994 (20.04.94) GB (71) Applicant (for all designated States except US): RPMS TECHNOLOGY LIMITED [GB/GB]; Commonwealth Building, Du Cane Road, London W12 0NN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): TAYLOR, Graham, Walter [GB/GB]; Dept. of Clinical Pharmacology, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN (GB). LACEY, Sandra, Lynn [GB/GB]; Dept. of Infectious Diseases and Bacteriology, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN (GB). (74) Agent: BASSETT, Richard; Eric Potter Clarkson, St. Mary's Court, St. Mary's Gate, Nottingham NG1 1LE (GB).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published With international search report.	
(54) Title: AMINOLINE-N-OXIDE ANTIMICROBIALS FOR USE AGAINST <i>H. PYLORI</i> INFECTIONS			
(57) Abstract			
<p>The use of a compound with structural formula (I) or the N-oxide thereof, wherein R₁ is H or lower alkyl and R₂ is C₁₋₂₀ alkyl or C₂₋₂₀ alkenyl with one or two unsaturated bonds, or a pharmaceutically acceptable salt, ester or salt of such ester or amide of such compounds, in the manufacture of a medicament for treating infection by microaerophilic bacteria, especially <i>Helicobacter pylori</i>. 2-heptyl-4-hydroxyquinoline, 2-nonyl-4-hydroxyquinoline, 2-nonenyl-4-hydroxyquinoline and their N-oxides are preferred.</p>			
		 <div style="text-align: right;">(I)</div>	

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

AMINOLINE-N-OXIDE ANTIMICROBIALS FOR USE AGAINST H. PYLORI INFECTIONS

The present invention relates to compounds that are effective in reducing or preventing infection by *Helicobacter pylori* or other microaerophiles.

- 5 In particular it relates to compounds that are useful in treating patients with gastric *Helicobacter pylori* infections and who suffer from gastric ulceration.

- 10 *Helicobacter pylori* (previously known as *Campylobacter pylori*) is recognised as the major cause of chronic gastritis in man. It is a Gram-negative microaerophile, that is to say it requires a low oxygen tension for growth (but cannot grow in the absence of oxygen) and a raised carbon dioxide level. Thus, typically, an oxygen concentration of about 3-15% and a CO₂ concentration of about 3-5% are required. The presence of *H.*
15 *pylori* in the pyloric antrum has been associated with duodenal ulcers and the organism has been linked to gastric ulcers and gastric carcinoma.

- Eradication of the *H. pylori* is often difficult because the bacterium is intrinsically resistant to a number of antibiotics, including vancomycin,
20 trimethoprim and the sulphonamides, and becomes resistant to other antibiotics that have been used against it, such as metronidazole and the macrolides.

- 2-Heptyl-4-hydroxyquinoline *N*-oxide is a lipoxygenase inhibitor and has
25 been proposed for use in treating bronchial asthma, allergic disorders, circulatory disorders and inflammations (EP 0 128 374).

- It has been shown previously that 2-heptyl-4-hydroxyquinoline *N*-oxide is an electron transport inhibitor in some bacteria and in mammalian
30 mitochondria and plant chloroplasts *in vitro*. 2-Heptyl-4-hydroxyquinoline

SUBSTITUTE SHEET (RULE 26)

N-oxide, produced by *Pseudomonas aeruginosa*, has also been shown to inhibit the growth of the Gram-positive bacteria *Staphylococcus aureus*, *Bacillus sphaericus*, *Bacillus subtilis* and *Listeria monocytogenes* (Machan *et al* (1992) *J. Antimicrobial Chemother.* 30, 615-623). This report
5 concluded that alkyl hydroxyquinoline *N*-oxides are only active against Gram-positive organisms; Gram-negative bacteria were found to be unaffected by these compounds.

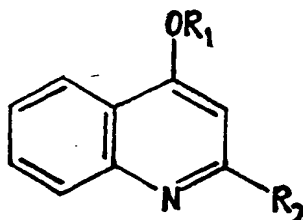
There has been a suggestion in the literature that some strains of
10 *Pseudomonas aeruginosa* inhibit *Helicobacter pylori*, the conclusion being that the *Ps. aeruginosa* could not be used for typing the *H. pylori*, which had been the hope. *Pseudomonas* are well known for secreting a variety of antibiotics (such as pyocyanin) and also non-specific proteases and so, especially in the light of the Machan *et al* disclosure that the said
15 quinoline compounds were inactive against Gram negative bacteria, there was nothing to suggest that these compounds were responsible for the anti-*Helicobacter* action. Indeed, the inhibition could have resulted simply from nutrient depletion.

20 Unexpectedly we have found that 2-alkyl-4-hydroxyquinolines and their *N*-oxides can inhibit the growth of Gram-negative microaerophiles, especially *Helicobacter pylori*. Thus, the present invention aims to provide new uses for, and formulations of, 2-alkyl-4-hydroxyquinolines and their *N*-oxides and new methods of treating or preventing infection by microaerophiles,
25 especially *Helicobacter pylori*.

A first aspect of the invention provides the use of a compound with the structural formula:

SUBSTITUTE SHEET (RULE 26)

3



or the *N*-oxide thereof, wherein R_1 is H or lower alkyl and R_2 is C_{1-20} alkyl or C_{2-20} alkenyl with one or two unsaturated bonds or a pharmaceutically acceptable salt, ester or salt of such ester or amide of
 5 such compounds, in the manufacture of a medicament for treating or preventing infection by Gram negative microaerophilic bacteria, especially *Helicobacter pylori*.

By "lower alkyl" we mean $-CH_3$, $-C_2H_5$, $-C_3H_7$ and $-C_4H_9$. It is preferred
 10 if R_1 is H. It is preferred if R_2 is a straight chain alkyl group or mono or di-unsaturated derivatives thereof. It is more preferred if R_2 is C_{5-12} alkyl or C_{5-12} alkenyl. It is still more preferred if R_2 is *n*-heptyl or *n*-nonyl or *n*-nonyl.

15 Conveniently, when R_1 is H a pharmaceutically acceptable ester or salt may be prepared as follows. Suitable esters can be made using acids or acid chlorides, for example, R_3COOH or R_3COCl wherein R_3 is an alkyl group which may contain an acidic or basic group. Preferably R_3 is $-CH_3$, $-C_2H_5$, $-C_3H_7$ or a carboxylic acid- or amino-derivative thereof.

20 Salts which may be conveniently used in therapy include physiologically acceptable base salts, for example, derived from an appropriate base, such as an alkali metal (eg sodium), alkaline earth metal (eg magnesium) salts, ammonium and NX_4^+ (wherein X is C_{1-4} alkyl) salts. Physiologically
 25 acceptable acid salts include hydrochloride, sulphate, mesylate, besylate,

SUBSTITUTE SHEET (RULE 26)

phosphate and glutamate.

Salts according to the invention may be prepared in conventional manner, for example by reaction of the parent compound with an appropriate base to form the corresponding base salt, or with an appropriate acid to form the corresponding acid salt.

It is further preferred if the compound is a 2-alkyl-4-hydroxyquinoline or its *N*-oxide. It is most preferred if the compound is 2-heptyl-4-hydroxyquinoline, 2-nonyl-4-hydroxyquinoline, 2-nonenyl-4-hydroxyquinoline or their *N*-oxides. The reduced compound is preferable to the *N*-oxide.

By "*Helicobacter pylori* infection" we mean any infection of man in which *H. pylori* can be isolated. Diseases of man that are associated with or caused by *H. pylori* infection include peptic ulcers, duodenal ulcers, gastric ulceration, mucosal-associated B-cell lymphomas and gastric carcinoma.

H. pylori is increasingly recognised as an important underlying cause of peptic ulcers. In addition, up to 95% of patients with duodenal ulcer have *H. pylori*-associated gastritis; *H. pylori*-negative patients with duodenal ulcers usually have some different aetiological factor, such as use of non-steroidal anti-inflammatory agents or pancreatitis.

The most common clinical features of benign gastric ulceration are pain and anaemia; the bleeding may be overt or occult. Spontaneous perforation of a gastric ulcer is less common than spontaneous perforation of a duodenal ulcer. It is very difficult to distinguish gastric ulcer pain from duodenal ulcer pain, pain due to gastric malignancy or other causes of dyspepsia. It has been suggested that *H. pylori* is a contributory cause

of gastric cancer, perhaps implicated in as many as 60% of cases. Gastric cancer is a major cause of cancer mortality.

5 Primary low grade B-cell lymphomas of the stomach have features of mucosal-associated lymphoid tissue (MALT). *H. pylori* is present in 90% of gastric MALT lymphomas. Given its close association with gastric MALT lymphoma, *H. pylori* might evoke immune responses and, in so doing, stimulate tumour growth. Thus, eradication of *H. pylori* should inhibit the growth of low-grade gastric lymphoma, and anti-*H. pylori* treatment should be given for this lymphoma as first line of treatment.

10 Biologically, gastric carcinoma is not a homogeneous entity and considerable differences in the aetiological forces involved have been identified for the several types so far recognised. Only some forms of gastric cancer appear to be associated with *H. pylori*.

20 The most common form linked to *H. pylori* arises in multifocal chronic atrophic gastritis (MAG). Epithelial cell damage is associated with *H. pylori* colonisation, in which a marked decrease of cytoplasmic mucin is seen where colonization is severe. Cell damage is followed by cell repair, in which the presence of the bacteria is associated with decrease in size and number of nuclei as well as their displacement to upper portions of the cytoplasm. From epidemiological and histopathological evidence, it is therefore clear that *H. pylori* is very much part of the chronic atrophic gastritis spectrum. MAG correlates very closely with the risk of gastric cancer; its prevalence is higher in populations at high cancer risk. In populations of low gastric cancer risk, MAG is very rare.

30 Thus, it is preferred if the medicament is used for treating gastric ulceration, mucosal-associated B-cell lymphomas, gastric carcinoma,

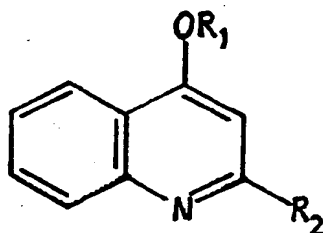
6

peptic ulcers or duodenal ulcers. The physician will readily diagnose the above diseases and others with which *H. pylori* infection is associated. *H. pylori* infection can be established using standard medical microbiological techniques.

5

The medicaments of the invention may also be useful in treating non-ulcer dyspepsia which is believed to involve *H. pylori* infection.

A second aspect of the invention provides a formulation comprising a
10 compound with the structural formula:



, or the *N*-oxide thereof, wherein R_1 is H or lower alkyl and R_2 is C_{1-20} alkyl or C_{2-20} alkenyl with one or two unsaturated bonds, or a pharmaceutically acceptable salt, ester or salt of such ester or amide of such compounds and a pharmaceutically acceptable carrier, wherein the
15 formulation is adapted for oral administration such that the compound is released in the stomach.

Preferred compounds of the formulation are the same as preferred
20 compounds of the first aspect of the invention.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (compound of the invention) with the carrier which
25 constitutes one or more accessory ingredients. In general the formulations

SUBSTITUTE SHEET (RULE 26)

are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

- 5 Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid
10 emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

- A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by
15 compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or
20 dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in
25 varying proportions to provide desired release profile.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

30

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include
5 flavouring agents.

It is preferred if the formulation provides preferential action in the stomach. A gavage using polyethylene glycol as the carrier may be suitable. The compounds of the invention appear to be acid stable. The
10 preferential action in the stomach may be achieved by providing for preferential release in the stomach or by providing for preferential retention in the stomach, for example by formulating the compound(s) in or on microspheres having adhesins specific for stomach mucus (eg sulphated groups) or stomach villi, or a moiety specific for *H. pylori*, for
15 example an antibody against an *H. pylori* adhesin. For example the K12 adhesin of *E. coli* may be used.

It is further preferred if the formulation contains a further compound effective at combating *H. pylori* infection such as tripotassium
20 dicitratobismuthate or metronidazole.

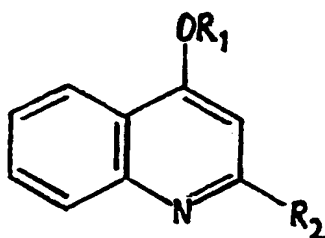
It is still further preferred if the formulation contains a compound effective at treating or preventing gastric or duodenal ulcers.

25 Suitable compounds include H_2 -receptor antagonists such as cimetidine, famotidine, nizatidine and ranitidine; selective anti-muscarinics such as pirenzepine; chelates and complexes such as bismuth chelate and sucralfate; prostaglandin analogues such as misoprostol; proton-pump inhibitors such as omeprazole; and other ulcer-healing drugs such as
30 carbenoxolone sodium.

SUBSTITUTE SHEET (RULE 26).

The formulation and dosage regime may provide for a local concentration in the site of action, for example the stomach, of above about 0.01 $\mu\text{g/ml}$, for example above about 0.5 $\mu\text{g/ml}$. Administration over a period of between 1 day and 10 weeks, for example about 3 weeks, should be sufficient to eradicate the bacteria.

A third aspect of the invention provides a method of treating a patient with an actual or suspected infection with a Gram negative microaerophilic bacterium, such as *Helicobacter pylori*, or to prevent such an infection comprising administering to the patient an effective amount of a compound with the structural formula:



, or the N-oxide thereof, wherein R_1 is H or lower alkyl and R_2 is C_{1-20} alkyl or C_{2-20} alkenyl with one or two unsaturated bonds, or a pharmaceutically acceptable salt, ester or salt of such ester or amide of such compounds and a pharmaceutically acceptable carrier.

Preferred compounds of the method of treatment are the same as the preferred compounds of the first aspect of the invention.

Further methods of treatment using the compounds of the invention include administering the compounds concomitantly with another compound effective at combating the infection (such as those described above) or a compound effective at treating or preventing gastric duodenal ulcers (such as those described above) or a combination thereof. For example the compounds of the invention may be administered to the

SUBSTITUTE SHEET (RULE 26)

patient on one day and the compound effective against ulcers may be administered to the patient on the next day.

5 The compounds may be made by either of the two general methods disclosed in EP 128 374, namely the method of Cornforth *et al* (1956 *Biochem. J.* 63, 124) or the method of Hirobe *et al* (1982 *Jap. Pharm. Soc. Lecture Summaries*, page 206), incorporated herein by reference.

10 The invention will now be described with reference to the following Examples and Figures wherein

15 Figure 1 shows the HPLC profile of the *Pseudomonas* anti-Helicobacter activity showing (a) A_{254} UV absorbance and (b) anti-bacterial activity expressed as the size of inhibition in the well plate assay. Activity elutes in fractions 25-40, and is well separated from pyocyanin and hydroxyphenazine (fractions 13 and 19, respectively). Fractions were collected after 4 minutes. Fraction 29 + 30 was in error doubly diluted.

20 Figure 2(a) shows the UV spectra taken in methanol of fraction 30 and Figure 2(b) that of fraction 25 both following HPLC purification.

25 Figure 3(a) and (b) shows fast atom bombardment mass spectra of fractions 30 and 31, respectively. Both spectra show intense protonated molecular ions ($M+H^+$) at m/z 272 and 270 together with smaller amounts of ions at m/z 240, 242. Note the ratio of m/z 272/270 increases from fraction 30 to fraction 31 consistent with partial separation of these two related materials by HPLC.

Example 1: Materials and methods used in identifying and testing compounds effective against *H. pylori*

Isolation and culture of bacteria

5

Helicobacter pylori: Strains of *H. pylori* were obtained from gastric biopsies of patients attending the gastrointestinal clinic at the Hammersmith Hospital, London. The biopsies were transported to the laboratory in a simple transport medium consisting of brain heart infusion
10 broth ((BHI) broth, Sigma, Poole, Dorset) and 5% (v/v) foetal calf serum (Gibco). The biopsies were plated on both selective (blood agar containing Skirrows supplement (Oxoid, Basingstoke, Hants, UK)) and non-selective (blood agar containing 2 µg/ml amphotericin (Oxoid)) media in the laboratory. The plates were incubated at 37°C on blood agar
15 medium containing amphotericin for approximately 3 days in Gas Pak anaerobic jars (Trademark, Oxoid) in the presence of a Campypak microaerophilic gas generating envelope (Trademark, Oxoid) and a palladium catalyst (Oxoid). Further subcultures were performed on blood agar plates containing amphotericin. (Also strains of *H. pylori* could be
20 preserved in BHI broth containing 10% glycerol under liquid nitrogen.) The identity of isolates was confirmed using Gram stain, oxidase urease and catalase. Each strain was tested for metronidazole sensitivity using a 5 µg metronidazole disc (Oxoid). *H. pylori* was also grown in broth culture in BHI broth plus 10% horse serum (Gibco).

25

Pseudomonas aeruginosa: A clinical isolate of *P. aeruginosa* was grown on blood agar plates and incubated at 37°C overnight. The bacteria were removed by scraping and resuspended in BHI. A heavy suspension was then used to inoculate 400 ml of BHI and incubated at 37°C overnight.

30

Assays for anti-Helicobacter activity

Well plate assay: Antibacterial activity post HPLC was detected using the well plate assay. A sterile swab was used to spread a three day old
5 broth culture of test strains of *H. pylori* over the surface of blood/amphotericin plates (see above). Four wells (7 mm diameter) were cut in each plate with a sterile metal punch and filled with 100 μ l of suitable samples (eg post HPLC) dissolved in methanol. A methanol control was also used. Plates were incubated as above (section 2.1) and
10 the zone of inhibition caused by test samples measured after 3 days.

Plate MIC

Three 100 ml bottles of Isosensitest agar (Trademark, Oxoid) were melted
15 by placing them into a pressure cooker for 1 hour, then placed into a water bath at 55°C until used.

A stock solution of 4-heptyl-2-hydroxyquinoline *N*-oxide (referred to hereinafter as *N*-oxide) was made by dissolving 3 milligrams (mg) of it in
20 3 ml of methanol, followed by a further 1.68 ml of methanol to give a final concentration of 32 microgram/millilitre (μ g/ml).

A series of sterile universal bottles were labelled; 32 μ g, 16 μ g, 8 μ g, 4
25 μ g, 2 μ g, 1 μ g and 0.5 μ g.

1 ml of stock solution was added to the bottles labelled 32 μ g and 16 μ g. Concentrations of 8, 4, 2, 1, and 0.5 μ g/ml were prepared by doubling dilutions from 16 μ g/ml. This was done in duplicate and stored in the
30 freezer until used.

10 ml of fresh horse serum and 40 μ l of amphotericin (50 mg) was added to each isosensitest broth prior to cooling slightly. 19 ml of this was aliquoted into each of the dilutions and poured straight away into appropriately labelled sterile petri dishes. A control plate was set up - this
5 contained no N-oxide.

Once the plates had solidified, three day old broth cultures of 5 strains of *H. pylori* were pipetted into the wells of a sterile multiinoculator and the positions of each strains noted. Using the multi-point inoculator these
10 strains were seeded onto the agar plates containing decreasing concentrations of N-oxide. After all the plates had been inoculated, they were incubated at 37°C for approximately 3 days, in Gas Pak anaerobic jars in the presence of a Campypak microaerophilic gas generating envelope and a palladium catalyst.

15

Extraction of Pseudomonas anti-Helicobacter activity

After incubation in broth, the *P. aeruginosa* culture was divided into universal bottles and centrifuged at 3000 revolutions per minute for 15
20 min. 200 ml of culture supernatant was poured into a separating funnel and extracted twice with 400 ml of chloroform. Excess chloroform was removed by rotary evaporation at <30°C. The product was transferred to a universal bottle with 2 x 1 ml of methanol; solvent was removed under nitrogen.

25

High Performance Liquid Chromatography

HPLC was carried out on a Waters dual pump instrument (Millipore, UK, Harrow). Chromatography was undertaken at 2 ml/min on a μ Bondapak
30 C₁₈ column in a solvent system. The solvent system used was a 35 min

linear gradient of acetonitrile:water:heptafluorobutyric acid (20:80:0.04-70:30:0.04). The column eluate was monitored in the UV at 280 nm. Fractions (1 ml) were collected, solvents were removed under vacuum and resuspended in methanol (0.5 ml) for the well plate assay. Authentic standards 1-hydroxyphenazine, pyocyanin and 4-heptyl-2-hydroxyquinoline were chromatographed under the same HPLC conditions. Before performing HPLC, and each time the solvent was replaced, a blank control was run.

10 Ultraviolet spectroscopy

UV spectroscopy was carried out in methanol on a Perkin Elmer 555 instrument. Scans were undertaken between 450-220 nm at 60 nm/min. A holmium filter was used to confirm wavelength assignments.

15

Mass spectrometry

Mass spectrometry (MS) was carried on a Finnigan 4500 instrument in either the fast atom bombardment and desorption electron impact modes or by gas chromatography-electron impact modes.

20

Fast Atom Bombardment Mass spectrometry: FAB-MS was carried out using an M-Scan ion gun and xenon (10 kilo volts) as the ionising species. Samples were dissolved in 25 μ l of methanol and 2 μ l loaded onto the glycerol matrix for analysis.

25

Desorption electron impact MS: Samples were loaded onto a rhenium filament and desorbed at 10 amps per second.

30 Gas chromatography-electron impact MS: GC was carried out on a

DB5 capillary column (Jones Chromatography, Hengoed, Wales) using helium as a carrier gas. After 1 min at 200°C the column temperature was raised by 20°C from 200°C to 300°C. Samples were injected in octane using a Grob injector in the spitless mode set at 250°C. The gas chromatography column was routed into the mass spectrometer operated in the electron impact mode (70 eV electron energy).

Chemical reduction

A crude chloroform extract of *P. aeruginosa* was dissolved in 1 ml of methanol and 1 ml of 1.9 Molar titanium trichloride in 2 Molar hydrochloric acid (Sigma) was added. This was left at room temperature for 2 h with regular gentle shaking. The reduced product was extracted with 2 x 5 ml of chloroform; the chloroform layer was washed with 10 ml of water to remove any water soluble impurities. Solvent was removed under nitrogen. Authentic 2-heptyl-4-hydroxyquinoline *N*-oxide (Sigma) was reduced in a similar manner.

Derivatisation

Trimethylsilylation was carried out with 100 µl of bistrifluoromethyl-trifluoroacetamide and left at room temperature for 18 hr. Excess reagent was removed under nitrogen.

Materials

All chemicals were obtained from Sigma Chemical Company, Poole, Dorset, UK. All solvents were obtained from BDH Limited, Poole, England. Gas jars, Palladium catalyst, were obtained from Oxoid, Basingstoke, Hampshire. 1-Hydroxyphenazine and Pyocyanin were

provided by Dr G.W. Taylor, Royal Postgraduate Medical School, London.

Example 2: Isolation and identification of compounds effective against
H. pylori

Pseudomonas strain PYO5 was used. The strain was cultured in BHI broth and activity extracted into chloroform. The conditions used to obtain the best separation in a single step were a linear gradient of acetonitrile:water:FBA (20:80:0.04-04-70:30:0.04). Figure 1 shows the HPLC UV and bioactivity profile of a *Pseudomonas* chloroform extract. Although 50 x 1 ml fractions were collected, they were bulked into 25 x 2 ml fractions to determine bioactivity. Activity was associated mainly with a UV absorbing peak in fraction 31+32 although bioactivity was observed in neighbouring fractions (Figure 1, Table 1). A large scale preparation of *P. aeruginosa* was obtained from 1.2 l of culture filtrate, and, after chloroform extraction, chromatographed on HPLC. Although the resolution was somewhat diminished, activity was again found to be associated with fractions 31+32.

Table 1: The zones of inhibition in the well plate assay against sensitive and resistant strains of *H. pylori* for the HPLC fractions of the *Pseudomonas* factor.

Fraction number	Metronidazole sensitive		Metronidazole resistant	
	Diameter (mm)*	Area (mm) ² *	Diameter (mm)*	Area (mm) ² *
1-18	-	-	-	-
19+20	-	-	-	-
21+22	-	-	-	-

5	23+24	-	-	-	-
	25+26	-	-	-	-
	27+28	13	276	14	308
	29+30	10	668	10	668
	31+32	23	414	26	817
10	33+34	17	342	17	414
	35+36	15	57	15	342
	37+38	4	40	4	57
	39+40	3	-	4	57
	41+42	-	-	-	-
15	43-50	-	-	-	-
	Methanol control	-	-	-	-

- 15 Data are expressed as ring diameter (or area) less the diameter (area of the well).

Each active fraction was examined by UV spectroscopy in methanol, and the data are summarised in Table 2. The major active fractions (31+32) showed two peaks at 313 and 327 nm (Figure 2a). Similar spectra were present in other fractions. Fractions 25,26 which were also active, showed a different spectrum, with the λ_{max} shifted bathochromically to 326,335 nm (Figure 2b).

25 Each fraction was examined by fast atom bombardment mass spectrometry, which is a method suitable for molecular weight determination of polar and thermally unstable compounds. The primary ionising beam was a cold-cathode discharge source producing argon ions with an energy between 8 and 10 KeV. In the ion chamber resonant

charge-transfer takes place between the argon ions and the sample in its glycerol matrix. The atom beam takes the place of the electron beam in a classical electron-impact ionization source. Samples of mass M are converted to protonated ($M+H^+$) and cationised ($M+Na^+$) species, with little fragmentation. The active fractions (29-32) all generated FAB mass spectra (Table 2). In fraction 30, ions were present at m/z 242 (at low intensity), m/z 270 and, the major ion, at m/z 272; these correspond with masses of 241, 269 and 271 (Figure 3a). The base peak in fraction 31 was at m/z 272, with some m/z 270; fraction 32 also contained a lesser amount of m/z 272 (Figure 3b). Thus it appears that the activity present in fractions 31 and 32 is strongly associated with a compound of mass 271 together with smaller amounts of a substance with mass 269. Although fractions 30 to 32 gave a similar UV spectra, it was clear from the HPLC-UV profile that two closely related substances were present: m/z 272 mainly in fraction 31 and m/z 270 mainly in fraction 30.

Table 2: Summary of the λ_{max} , A_{max} and FAB-MS derived molecular weights for fractions 20-23 of the final HPLC conditions.

Fraction number	λ_{max}	A_{max}	Chemical class* (HQN/HQNO)	Molecular weight (FAB-MS)
20-23	****	-	-	242,270
24	313,327	0.3	HQN	244
25	313,327	0.7	HQNO	244,260
26	326,335	0.48	HQNO	242,260, 270
27	313,327	0.25	HQN	242,270
28	****	-	-	242,270
29	307,327	0.35	?	242,270
30	313,327	1.28	HQN	242,270

SUBSTITUTE SHEET (RULE 26)

31	313,327	0.98	HQN	242,272
32	313,327	0.34	HQN	242,272

*** insufficient material.

- 5 * HQN = 2-alkyl-4-hydroxyquinoline like spectrum. HQNO: 2-alkyl-4-hydroxyquinoline N-oxide like spectrum.

10 The UV absorbance and FAB-MS of fractions 24, 27, 31, 32 and 33 corresponded to a quinoline, whilst the fractions 25 and 26 corresponded with an N-oxide.

15 The UV absorbance and mass spectrometric data are consistent with the presence of known secondary metabolites of *P. aeruginosa*, 2-nonyl-4-hydroxyquinoline and 2-nonenyl-4-hydroxyquinoline. The related material, 2-heptyl-4-hydroxyquinoline N-oxide is available commercially from Sigma. It is used as a streptomycin antagonist, and inhibits NADH oxidation by mitochondria. The UV spectrum in methanol of this material exhibited absorbances at λ_{\max} 326 nm ($\epsilon = 9040$) with a second peak at 335 nm ($\epsilon = 8750$). This spectrum is similar to that found in HPLC fraction 25 (Figure 2b) from the *Pseudomonas* preparation. The FAB mass spectrum of the N-oxide showed ions at m/z 260 ($M+H^+$) and 244 (loss of oxygen). Again, fraction 25 of the purified *Pseudomonas* extract behaved in a similar manner (Table 2).

25 Treatment of the authentic 2-heptyl-4-hydroxyquinoline N-oxide with titanium trichloride resulted in the formation of the reduced material, 2-heptyl-4-hydroxyquinoline. The absorption spectrum of this material ($\lambda_{\max} = 313$ nm and 327 nm, $\epsilon_{313} = 9120$ and $\epsilon_{327} = 9250$) is consistent with that previously reported for 2-heptyl-4-hydroxyquinoline. The UV spectrum was similar to that found in a number of HPLC fractions from the *Pseudomonas* preparation; in particular, the major UV absorbing

30

SUBSTITUTE SHEET (RULE 26)

species (fractions 30+31) gave identical UV spectra. Mass spectrometry was used to confirm the identity of the reduced material. The FAB mass spectrum confirmed that the N-oxide had been quantitatively reduced (yielding $M+H^+$: m/z 244). GC-MS was performed on the reduced quinoline using electron impact ionisation; the compound eluted after 8:02 min and generated a mass spectrum with intense ions at m/z 243 (M^+), 172 ($-C_5H_{11}$) and 159 ($-C_6H_{12}$). Derivatisation with bistrifluoromethyl-trifluoroacetamide converted the quinoline into its mono-trimethylsilyl derivative, which eluted at 5:50 min generating a characteristic mass spectrum. These data confirmed the chemical identity of the reduced material as 2-heptyl-4-hydroxyquinoline. On chromatographing the quinoline on HPLC, it eluted as a single UV absorbing peak at 25 min.

Samples of both authentic 2-heptyl-4-hydroxyquinoline-N-oxide and the reduced quinoline were examined in the well plate assay. These compounds were active against both metronidazole sensitive and resistant strains of *H. pylori*, requiring approx 0.1 μg to cause significant inhibition (> 22 mm zone diameter, Tables 3 and 4). In comparison, a 5 μg disc of metronidazole gave a 19 mm zone of inhibition for the sensitive strain only. The plate MIC values for the quinoline and its N-oxide against *H. pylori* were also determined against three strains of the organism as at most 0.5 $\mu g/ml$, and probably < 0.015 $\mu g/ml$.

Table 3: Inhibition of *H. pylori* growth in the well plate assay by 2-heptyl-4-hydroxyquinoline N-oxide.

Amount of C ₇ -HQNO added (μg in 100 μl)	Average zone of inhibition (mm)	
	Metronidazole sensitive*	Metronidazole resistant
0.1	22	19
1	32	33
10	38	36
100	40	41

Table 4: Inhibition of *H. pylori* growth in the well plate assay by 2-heptyl-4-hydroxyquinoline.

Amount of C ₇ -HQN (μg in 100 μl)	Average zone of inhibition (mm)	
	Metronidazole sensitive	Metronidazole resistant
0.075	25	27
0.75	28	29
7.5	40	38
75	> 40	> 40

As the concentration of authentic C₇-HQN or the N-oxide increased the zone of inhibition also increased and there was no significant difference in activity between metronidazole sensitive and metronidazole resistant strains. *For comparison, a 5 μg disc of metronidazole generated a zone of inhibition of 19 mm.

The inhibitory effect of native and reduced factor extracted from *Pseudomonas* was also determined (Table 5).

Table 5: Inhibitory effect (n = 2) of native and reduced *Pseudomonas* factor on both metronidazole sensitive (+) and resistant (-) *H. pylori*.

	Native pseudomonas factor		Reduced factor	
	Metronidazole sensitivity			
	+	-	+	-
5 Zone of inhibition (mm)	33,40	35,36	36,41	34,48

10 In summary, the anti-*Helicobacter* activity of *P. aeruginosa* has been characterised as 2-nonyl-4-hydroxyquinoline and its nonenyl analogue. This material is active against metronidazole sensitive and resistant strains of *H. pylori*. Using synthetic 2-heptyl-4-hydroxyquinoline, the MIC of this class of chemicals has been found to be well below that of other antibiotics (Table 6).

15

Table 6: MIC₉₀ values of various antibacterials.

Antibacterial	MIC ₉₀ (μg/ml)
2-heptyl-4-hydroxyquinoline	<0.015
Penicillin; ampicillin	<0.5
Flucloxacillin; aztreonam	2
Nitrofurantoin	0.5
Furazolidine	0.25
Tetracycline	0.25
Rifampicin	1

Example 3: Synthesis of 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide

Methyl 3-oxodecanoate (48 g) was added dropwise to a stirred suspension of powdered sodium (5.52 g) in toluene (100 ml) and benzene (200 ml) under N₂. When the sodium was dissolved, *o*-nitrobenzoyl chloride was added slowly with stirring and cooling. Next day dilute H₂SO₄ was added and the product (84 g) was isolated in the normal manner with the help of some more benzene. It was then boiled with dilute H₂SO₄ (500 ml of 33%, w/w) and dioxan (68 ml) for 7 hr. The cooled mixture was extracted with ether, and this, after washing with aqueous NaHCO₃, was extracted with 0.5N-NaOH (1 l) in seven portions which were acidified immediately after separation. The acidic product was recovered by means of ether; it was treated with cold light petroleum (bp 40-60°), and a residue (8 g) of *o*-nitrobenzoic acid was removed. The light-petroleum-soluble product was a red oil (30 g). A portion (5 g) was added to a mixture of SnCl₂·2H₂O (14 g) and acetic acid (45 ml), with sufficient dry HCl to effect dissolution. The mixture became warm; passage of hydrogen chloride was continued until it had cooled. After an hour the mixture was poured into water and extracted with chloroform. The

SUBSTITUTE SHEET (RULE 26)

chloroform was washed with water (emulsions were broken by filtration) and with aqueous NaHCO_3 and evaporated. The residue on trituration with ethyl acetate gave the crystalline *N*-oxide (1.5 g), which was purified by recrystallization from ethanol:colourless leaflets, mp 158-160°. The compound is also available from Sigma (Poole, Dorset, UK).

Example 4: Synthesis of 2-*n*-nonyl-4-hydroxyquinoline *N*-oxide

Methyl 3-oxododecanoate (20 g) was condensed, as described above, with *o*-nitrobenzoyl chloride, and the product was hydrolysed with dioxan-sulphuric acid by boiling for 18 hr. An ethereal extract of the hydrolysed product was shaken with saturated aqueous cupric acetate, when *copper* 1-(2-nitrophenyl)dodecane-1:3-dionate separated; a portion crystallized from ethanol in blue-grey needles, mp 149.5-150.5° [Found: N, 4.1 ($\text{C}_{18}\text{H}_{24}\text{O}_4\text{N})_2\text{Cu}$ requires N, 4.0%], the rest was decomposed by shaking with ether and dilute sulphuric acid. After evaporation of the ether the residue was crystallized from light petroleum (bp 40-60°) to give 1-(2-nitrophenyl)dodecane-1:3-dione (V; $\text{R} = [\text{CH}_2]_8\text{CH}_3$) (8.7 g). A sample after two more crystallizations formed plates, mp 44°. (Found: C, 67.7; H, 7.5; N, 4.4. $\text{C}_{18}\text{H}_{25}\text{O}_4\text{N}$ requires C, 67.7; H, 7.8; N, 4.4%). The diketone (7.6 g) was reduced with SnCl_2 , as described above; but the reaction mixture was left overnight before dilution and became very dark. The product was purified by three crystallizations from ethanol; the *N*-oxide formed colourless leaflets (2.5 g), mp 148-149°. (Found: C, 75.0; H, 8.6; N, 5.1. $\text{C}_{18}\text{H}_{25}\text{O}_2\text{N}$ requires C, 75.3; H, 8.7; N, 4.9%.) The ultraviolet absorption in 0.001 N-NaOH was identical with that of the heptyl analogue.

Example 5: Synthesis of 2-n-undecyl-4-hydroxyquinoline N-oxide

Methyl 3-oxotetradecanoate (11.95 g, mp 29-30°), purified by low-temperature crystallization from methanol, was condensed as described
5 above with *o*-nitrobenzoyl chloride, and the product was hydrolysed by boiling for 17 hr with dilute sulphuric acid and dioxan. *Copper 1-(2-nitrophenyl)-tetradecane-1:3-dionate* was prepared as described above. A sample crystallized from ethanol in blue-grey needles, mp 150-151° (Found: N, 4.1. $C_{20}H_{28}O_4N_2$ requires N, 3.7%); from the remainder. 1-
10 (2-nitrophenyl)tetradecane-1:3-dione (V; R = $[CH_2]_{10}.CH_3$) was prepared as above and was crystallized from light petroleum (bp 40-60°): yield, 4 g, mp 53-54°. A sample on further crystallization formed almost colourless plates, mp 53.5-54.5°. (Found: C, 69.1, 69.2; H, 8.2, 8.3; N, 4.4. $C_{20}H_{29}O_4N$ requires C, 69.2; H, 8.4; N, 4.0%.) The diketone
15 (3 g) was reduced with $SnCl_2$ as described for the heptyl analogue; however, the chloroform extract of the diluted mixture was thoroughly shaken with dilute sulphuric acid before washing with water. In this way, emulsions caused by separation of tin oxides were avoided. The product on crystallization from ethanol gave leaflets (1.8 g), mp 144-146°. Two
20 further crystallizations gave the *N-oxide* in colourless shining leaflets, mp 148.5-149.5°. (Found after drying at 80°: C, 76.4; H, 8.8; N, 4.8. $C_{20}H_{29}O_2N$ requires C, 76.2; H, 9.2; N, 4.4%.)

2-alkyl-4-hydroxyquinoline *N*-oxides of different chain lengths and degrees
25 of saturation can be produced using the above methods by substituting the appropriate methyl 3-oxoalkanoate.

The corresponding 2-alkyl-4-hydroxyquinolines can be readily synthesised from the *N*-oxides using $TiCl_3/HCl$ as the reducing agent as described in
30 the Examples.

SUBSTITUTE SHEET (RULE 26)

Example 6: Pharmaceutical formulations

The following examples illustrate pharmaceutical formulations according to the invention in which the active ingredient is a compound of the formula of the first aspect of the invention.

Formulation 1: Tablet

	Active ingredient	100 mg
10	Lactose	200 mg
	Starch	50 mg
	Polyvinylpyrrolidone	5 mg
	Magnesium stearate	<u>4 mg</u>
		359 mg

15

Tablets are prepared from the foregoing ingredients by wet granulation followed by compression.

The following formulations 2 and 3 are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

20

27

Formulation 2

	<u>mg/tablet</u>	<u>mg/tablet</u>
(a) Active ingredient	250	250
(b) Lactose B.P.	210	26
5 (c) Povidone B.P.	15	9
(d) Sodium Starch Glycolate	20	12
(e) Magnesium Stearate	<u>5</u>	<u>3</u>
	500	300

10 Formulation 3

	<u>mg/tablet</u>	<u>mg/tablet</u>
(a) Active ingredient	250	250
(b) Lactose	150	-
(c) Avicel PH 101*	60	26
15 (d) Povidone B.P.	15	9
(e) Sodium Starch Glycolate	20	12
(f) Magnesium Stearate	<u>5</u>	<u>3</u>
	500	300

20 Formulation 4

	<u>mg/tablet</u>
Active ingredient	100
Lactose	200
Starch	50
25 Povidone	5
Magnesium stearate	<u>4</u>
	359

The following formulations, 5 and 6, are prepared by direct compression
 30 of the admixed ingredients. The lactose used in formulation E is of the

SUBSTITUTE SHEET (RULE 26)

28

direction compression type.

Formulation 5

	<u>mg/capsule</u>
5 Active Ingredient	250
Pregelatinised Starch NF15	<u>150</u>
	400

Formulation 6

	<u>mg/capsule</u>
10 Active Ingredient	250
Lactose	150
Avicel *	<u>100</u>
	500

15

Formulation 7 (Controlled Release Formulation)

The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone followed by the addition of magnesium stearate and compression.

20

	<u>mg/tablet</u>
(a) Active Ingredient	500
(b) Hydroxypropylmethylcellulose (Methocel K4M Premium)*	112
25 (c) Lactose B.P.	53
(d) Povidone B.P.C.	28
(e) Magnesium Stearate	<u>7</u>
	700

30 Drug release takes place over a period of about 6-8 hours and was

SUBSTITUTE SHEET (RULE 26)

complete after 12 hours.

Formulation 8

- 5 A capsule formulation is prepared by admixing the ingredients of Formulation 5 above and filling into a two-part hard gelatin capsule. Formulation 9 (*infra*) is prepared in a similar manner.

Formulation 9

10		<u>mg/capsule</u>
	(a) Active ingredient	250
	(b) Lactose B.P.	143
	(c) Sodium Starch Glycolate	25
	(d) Magnesium Stearate	<u>2</u>
15		420

Formulation 10

		<u>mg/capsule</u>
	(a) Active ingredient	250
20	(b) Macrogol 4000 BP	<u>350</u>
		600

- 25 Capsules are prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling the melt into a two-part hard gelatin capsule.

30

Formulation 11

	<u>mg/capsule</u>
Active ingredient	250
Lecithin	100
5 Arachis Oil	<u>100</u>
	450

Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

10

Formulation 12 (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin capsule.

	<u>mg/capsule</u>
(a) Active ingredient	250
20 (b) Microcrystalline Cellulose	125
(c) Lactose BP	125
(d) Ethyl Cellulose	<u>13</u>
	513

Example 13: Syrup Suspension

	Active ingredient	0.2500 g
	Sorbitol Solution	1.5000 g
5	Glycerol	2.0000 g
	Dispersible Cellulose	0.0750 g
	Sodium Benzoate	0.0050 g
	Flavour, Peach 17.42.3169	0.0125 ml
	Purified Water q.s. to	5.0000 ml

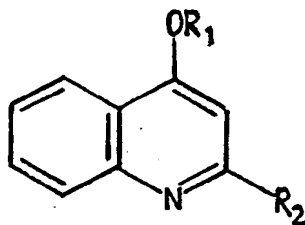
10

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol solution added. The active ingredient is added and dispersed. In the glycerol is dispersed the thickener (dispersible cellulose). The two dispersions are mixed and made up to the required volume with the

15 purified water. Further thickening is achieved as required by extra shearing of the suspension.

CLAIMS

1. The use of a compound with the structural formula:



- or the *N*-oxide thereof, wherein R₁ is H or lower alkyl and R₂ is C₁₋₂₀ alkyl or C₂₋₂₀ alkenyl with one or two unsaturated bonds, or a pharmaceutically acceptable salt, ester or salt of such ester or amide of such compounds, in the manufacture of a medicament for treating or preventing infection by Gram negative microaerophilic bacteria, especially *Helicobacter pylori*.
2. The use of a compound according to Claim 1 wherein R₁ is H.
3. The use of a compound according to Claim 1 or 2 wherein R₂ is a straight-chain alkyl group or mono- or di-unsaturated derivative thereof.
4. The use of a compound according to any one of Claims 1 to 3 wherein R₂ is *n*-heptyl, *n*-nonyl or *n*-nonyl.
5. The use of a compound according to any one of the preceding claims wherein the infection is associated with gastric ulcers, mucosal-associated B-cell lymphomas, gastric carcinomas, peptic ulcers or duodenal ulcers.
6. A formulation comprising a compound as defined in any one of Claims 1 to 4 and a pharmaceutically acceptable carrier wherein the

SUBSTITUTE SHEET (RULE 26)

formulation is adapted for oral administration such that the said compound is released in the stomach.

7. A formulation according to Claim 6 which provides preferential
5 action in the stomach.
8. A formulation according to Claim 6 or 7 further comprising a
further compound effective at combating infection by microaerophilic
bacteria.
- 10
9. A formulation according to Claim 8 wherein the agent is
tripotassium dicitratobismuthate or metronidazole.
10. A formulation according to any one of Claims 6 to 9 further
15 comprising a compound effective at treating or preventing gastric or
duodenal ulcers.
11. A formulation according to Claim 10 wherein the compound
effective against ulcers is selected from the group consisting of cimetidine,
20 famotidine, nizatidine, ranitidine, pirenzapine, sucralfate, misoprostol,
omeprazole and carbenoxolone.
12. A method of treating a patient with an actual or suspected infection
by Gram negative microaerophilic bacteria, especially *Helicobacter pylori*,
25 or to prevent such an infection comprising administering to the patient an
effective amount of a compound as defined in any one of Claims 1 to 4.
13. A method of treating a patient according to Claim 12 further
comprising administering concomitantly or separately a compound
30 effective at combating Gram negative microaerophilic bacterial infection

or a compound effective at treating or preventing gastric or duodenal ulcers or a combination thereof.

SUBSTITUTE SHEET (RULE 26)

1/3

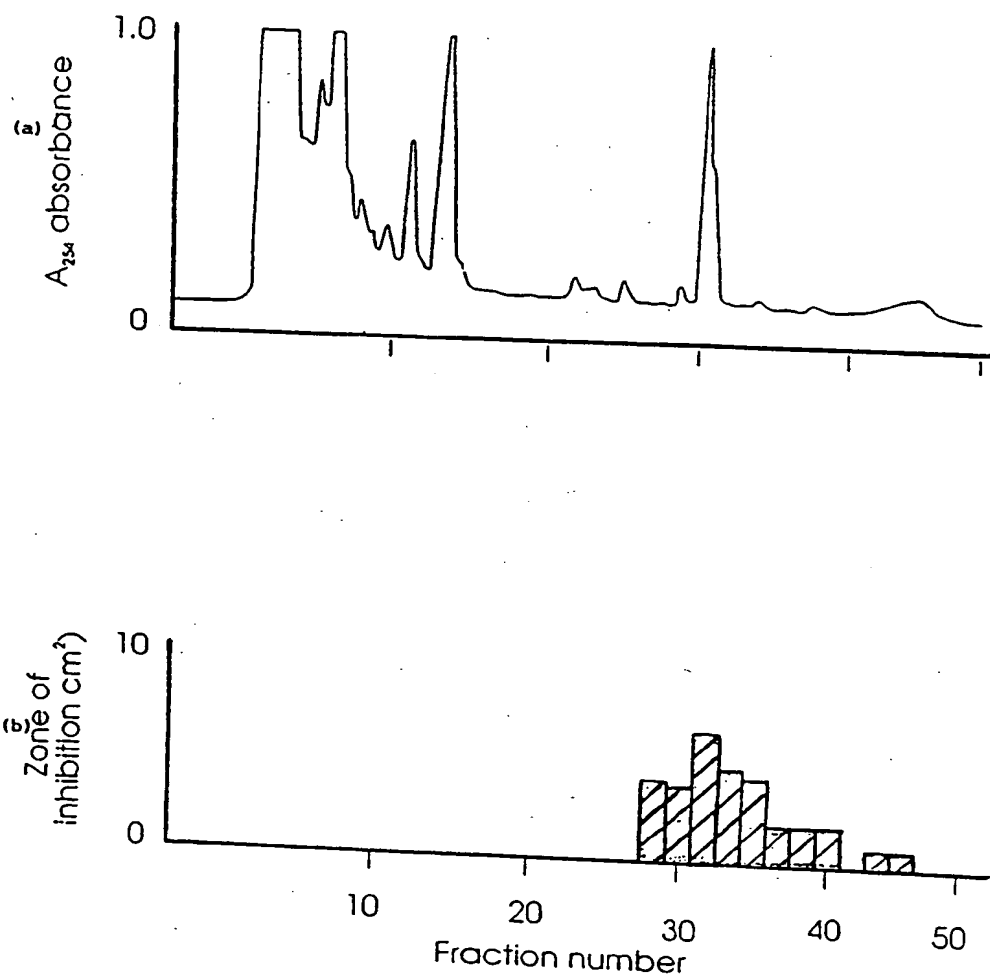


Figure 1

SUBSTITUTE SHEET (RULE 26)

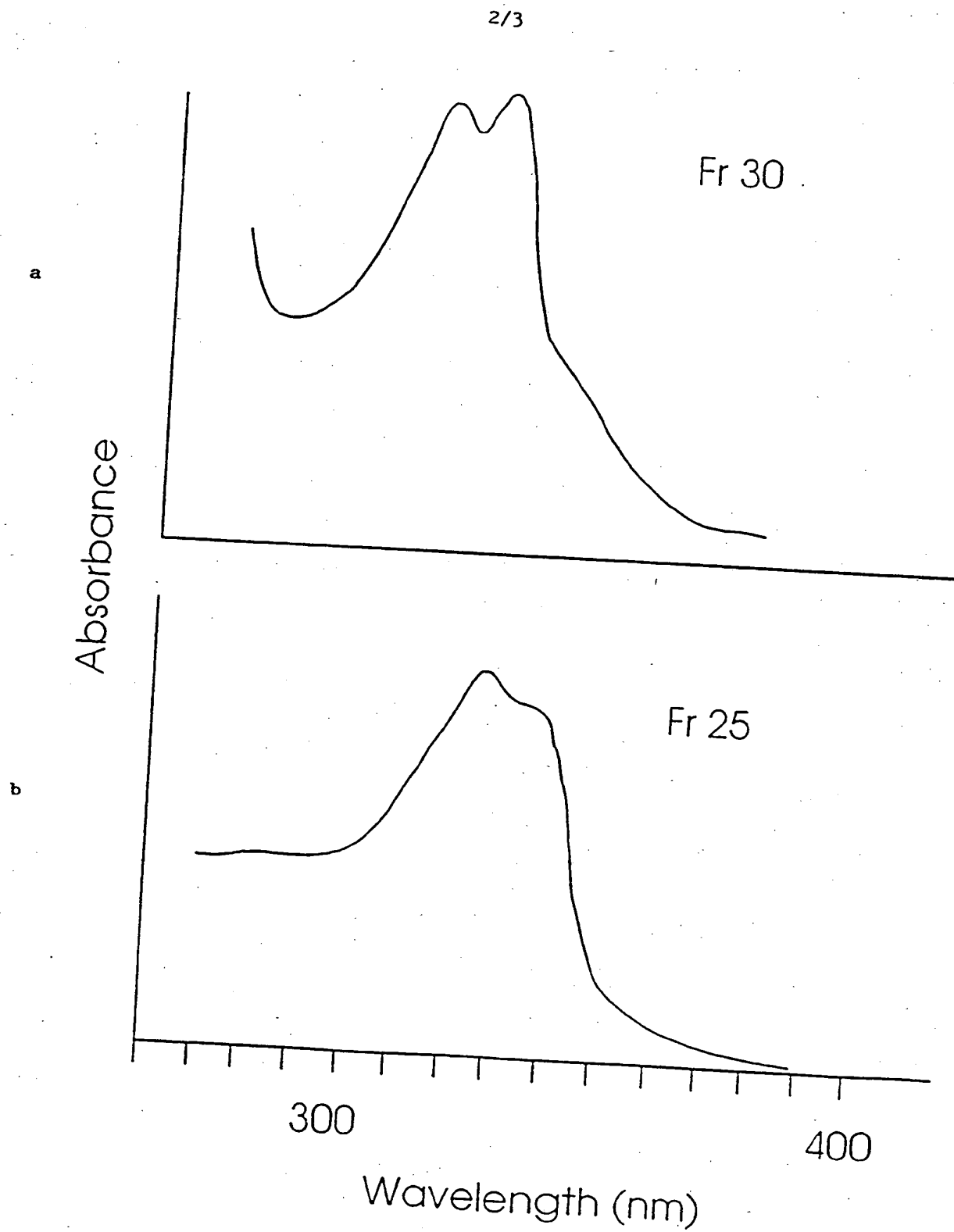


Figure 2

SUBSTITUTE SHEET (RULE 26)

3/3

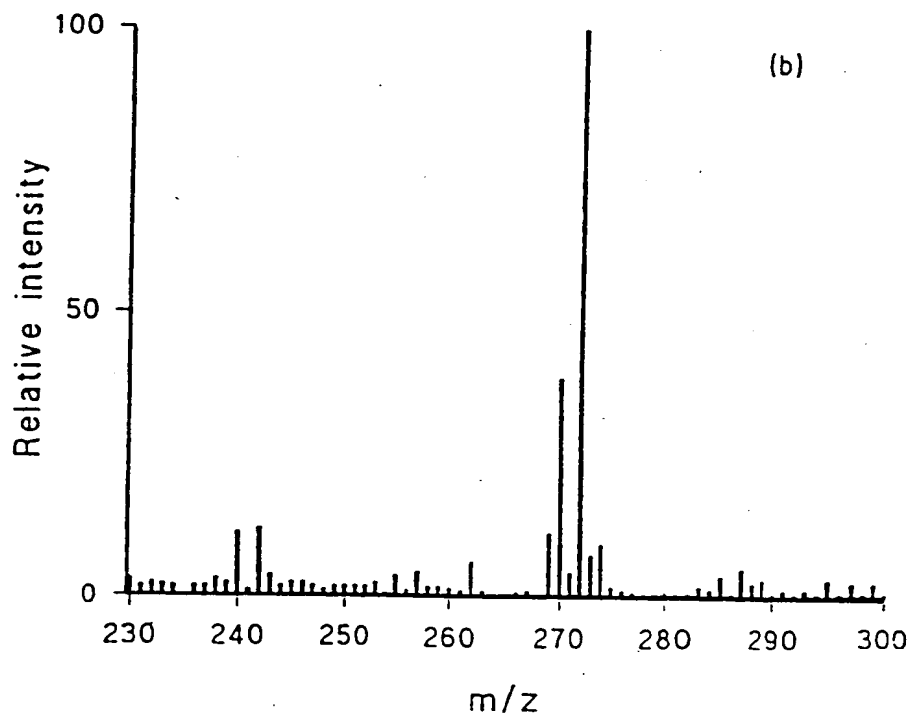
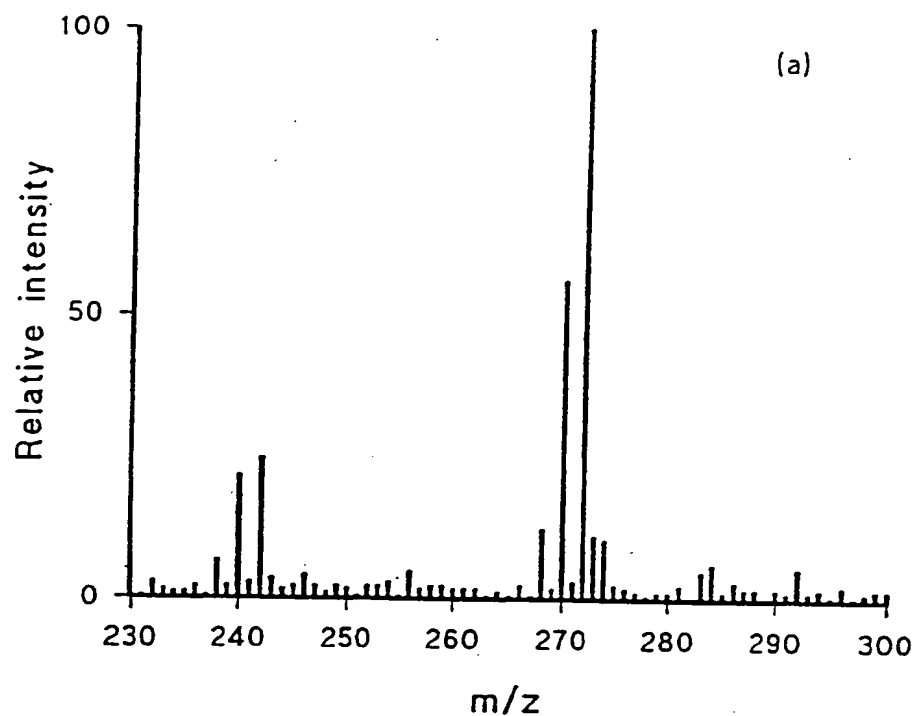


Figure 3

SUBSTITUTE SHEET (RULE 26)

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A, 0 128 374 (KYOWA LTD) 19 December 1984 cited in the application ---	
A	DATABASE MEDLINE Knight-Ridder accession number 08796306, see abstract & NIPPON RINSHO, vol. 51, no. 12, 1993 pages 3159-3162, S. KIMURA ET AL. 'Leukotriene levels of Helicobacter pylori-infected gastric mucosa.' -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- '&' document member of the same patent family

Date of the actual completion of the international search

18 July 1995

Date of mailing of the international search report

26.07.95.

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Klaver, T